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DIFFERENTIAL MOLYBDENUM MEDIUM  
FOR PLAGUE AND  
PSEUDOTUBERCULOSIS BACTERIA

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Translated by Sp/6 Charles T. Ostertag Jr.

Differential Molybdenum Medium for Plague and Pseudotuberculosis Bacteria

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For the differential diagnosis of plague and pseudotuberculosis bacteria, which have similar biological properties, we used a medium suggested by Andreeva for the recognition of various representatives of the intestinal group. We tested the medium both in a liquid and solid form. As is known, rosolic acid and ammonium molybdate are the indicators in the medium. According to Andreeva, ammonium molybdate is the indicator of the oxidation-reduction potential of the medium ( $rH_2$ ) and rosolic acid is used to judge the pH of the medium. The proposed differential medium is based on the fact that during the growth of the pseudotuberculosis microbe, the nutrient medium acquires an alkaline reaction and during the growth of the plague microbe - an acid one, as a result of which the color of the medium changes.

The medium contains:

100 ml of plain broth or agar, pH = 7.3  
1% glucose  
rosolic acid (5% alcohol solution) 0.2%  
(the medium becomes red)  
ammonium molybdate 0.4%  
(the medium acquires a yellow pigmentation)  
1% glycerin  
1% urea  
sodium carbonate ( $Na_2CO_3$ ) 17.5 ml of a 10% solution to 1000 ml  
of medium

The medium was sterilized one time by using free-flowing steam for 30 minutes (autoclave or Koch's apparatus). In prepared form after sterilization, the medium had a red color.

The growth of 82 museum strains and 79 strains of pseudotuberculosis bacteria was studied in this medium.<sup>1</sup>

A two day culture of bacteria was inoculated in the specified medium. The cultures were incubated at 28°C. The growth result was observed after 16, 20, 24 and 48 hours, then daily for 3 weeks. The medium in which plague cultures were inoculated discolored completely in 20 - 24 hours and acquired the pigmentation of plain broth with a somewhat yellowish hue.

Subsequently, depending on the properties of the strain, the yellow color of the medium acquired a rose-colored pigmentation of various intensity (the period of observation was 21 days). After 20 - 24 hours, the medium in which pseudotuberculosis cultures were inoculated was the same shade of red or even brighter than in the control.

Media containing strains of the plague bacteria being studied were virulent, weakly virulent and avirulent. Media containing strains of the pseudotuberculosis bacillus were dissociated, strains in the R- and S-form and 5 strains obtained as a result of the mutability of the plague microbe (Saratov "Mikrob" Institute). All strains were isolated from various hosts and had a different storage period.

Out of 79 pseudotuberculosis strains, in 24 we were able to follow hourly the changes in color of the liquid differential media in relation to the growth of the culture.

Hourly Changes in the Color of a Liquid Differential Medium in Relation to the Growth of Pseudotuberculosis Bacilli

Overall number of strains	Number of strains changing the color of the medium in various periods (in hours)								
	Lightly discolored				More intensely discolored		Turns pink	Turns red	
	6	7	8	9	10	11	12	24	48
29	5	22	28	29	29	29	29	29	29

Table 1

From table 1 it is apparent that after 6 hours of growth of the pseudotuberculosis microbe, the medium discolored slightly due to acidification. The discoloration continued for 3 more hours and then for 2 hours the reaction didn't change. After 12 hours of growth, alkalization of the medium began which was detected according to the degree that it was turning red. By 24 hours of growth the medium acquired an intense red pigmentation which was preserved up to the end of the observation.

The rapidity of changes in reaction (coloration) of the medium depended on the properties of the strains, on the state (liquid or solid) of the medium, and on the inoculation dose of the culture. For example, upon inoculation of a culture, one loop in volume (1 mm in diameter), the color of a liquid medium changed in 16 - 20 hours while with the inoculation of a smaller amount of microbial cells the color of the medium changed when a sufficient quantity of bacteria accumulated which were capable of changing the reaction of the medium.

According to Andreeva, during tubing it is possible to use unsterilized test tubes since rosolic acid is an inhibitor of saprophytic flora. The medium is not sensitive to the effect of light and is well preserved.

For comparison we conducted parallel inoculations on the medium of Timofeyeva and Golovacheva. As a result it was disclosed that the proposed medium (particularly the liquid one) provided a brighter, sharper change of color than Timofeyeva's medium, which eases the calculation of results.

It is possible to use the medium recommended by us extensively under expeditionary conditions due to the fact that it preserves well, saprophytic microflora do not develop on it, and after 20 - 24 hours of growth it is possible to differentiate plague bacteria from pseudotuberculosis bacteria.

Besides this, we observed that the addition of ammonium molybdate,  $(\text{NH}_4)_2\text{MoO}_4$ , to a plain nutrient medium (meat-peptone agar or broth) promoted the growth of single microbial cells of plague bacteria just as well as the addition of other known growth stimulators (sodium sulfite, blood).

Table 2 (at end of article)

From table 2 it is apparent that with the addition of ammonium molybdate to plain meat-peptone agar, almost the same results are obtained as with the addition of blood or sodium sulfite. The morphology of colonies remains typical and doesn't differ from their morphology on agar with sodium sulfite.

#### Conclusions

1. The recommended liquid molybdenum medium made it possible, in the course of 20 - 24 hours, to differentiate plague bacteria from pseudotuberculosis bacteria.
2. The medium preserved itself well enough that it could have been used a month after it was prepared.
3. The medium may be recommended for use under expeditionary conditions during epizootiological inspections.
4. When inoculated in small doses, ammonium molybdate stimulated the growth of plague bacteria which were being cultivated on meat-peptone media analogous to other known stimulators of growth of the plague microbe (sodium sulfite, blood).

Footnote 1. Of the 79 pseudotuberculosis strains, 50 freshly isolated strains were investigated in the laboratory of the Leningrad Anti plague Port and Municipal Observation Station (Chief - L. I. Gur'yanova).



#### Literature

Andreeva, G. V., Labor. Delo (Laboratory Affairs), 1959, No. 2, p. 40.

Andreeva, G. V., J. Microbiol., 1959, No. 2, p. 70.

Timofeeva, L. A., Golovacheva, V. Ya., Proceedings of the Irkutsk Scientific-Research Antiplague Institute of Siberia and the Far East, 1957, t. 14, p. 46.

Timofeeva, L. A., Aparin, G. P., Golovacheva, V. Ya., Ibid., t. 15, p. 123.

(The following English summary appears with the Russian Original)

#### Differential Molybden Medium for Plague and Pseudotuberculosis Bacilli

N.E. Gubina, T.T. Voronina and N.P. Pobivantseva

The medium, suggested by Andreeva, for differentiating the intestinal group of microbes, was tested for the differentiation of plague and pseudotuberculosis bacilli. 82 museum strains of *B. pestis* and 79 strains of *B. pseudotuberculosis rodentium* were studied. The medium was bright red in colour prior to the inoculation. Plague bacilli decolourized the medium in 20 - 24 hours, whereas pseudotuberculosis bacilli did not affect its colour.

Similar to such stimulants as sodium sulfite or blood, ammonium molybdate stimulated the growth of *B. pestis* when added to the nutritive medium to be tested.

Comparative growth of B. pestis EB on meat-peptone agar with the addition of ammonium molybdate and other growth stimulators		Number of colonies upon inoculation of microbes in various doses (in number of microbial cells)									
Name of nutrient medium	Number of strains	5				50				500	
		In all expts	Aver- age	In all expts	Aver- age	In all expts	Aver- age	In all expts	Aver- age	In all expts	Aver- age
Meat-peptone agar, plain	5	0	0	0	0	0	0	0	0	2	0.4
Meat-peptone agar + ammonium molybdate	5	19	3.6	189	37.8	1613	322.6	solid growth			
Meat-peptone agar + sodium sulfite	5	15	3	169	33.8	1384	276.8	"	"	"	"
Meat-peptone agar + lysed blood	5	15	3	205	41	1736	347.2	"	"	"	"

Table 2